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Evaluation and statistical optimization of a method for methylated cell-free fetal DNA extraction from maternal plasma

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Abstract

Purpose Methylated cell-free fetal DNA (cffDNA) in maternal plasma can potentially be used as a biomarker for accurate noninvasive prenatal testing (NIPT) of fetal disorders. Recovery and purification of cffDNA are key steps for downstream applications. In this study, we aimed to developed and evaluated different aspects of an optimized method and compared its efficiency with common methods used for extraction of methylated cffDNA.

Methods Single factor experiments, Plackett-Burman (PB) design, and response surface methodology (RSM) were conducted for conventional Triton/Heat/Phenol (cTHP) method optimization. The total cell-free DNA (cfDNA) was extracted from pooled maternal plasma using the optimized method called the Triton/Heat/Phenol/Glycogen (THPG), cTHP method, a column-based kit, and a magnetic bead-based kit. In the next step, methylated cfDNA from the extracted total cfDNA was enriched using a methylated DNA immunoprecipitation (MeDIP) kit. Real-time quantitative polymerase chain reaction was performed on the RASSF1 gene and hyper region to determine the genomic equivalents per milliliter (GEq/ml) values of the methylated cfDNA and cffDNA, respectively.

Results The optimum values of the significant factors affecting cfDNA extraction from 200 μl of plasma were 3% SDS, 1% Triton X-100, 0.9 μg/μl glycogen, and 0.3 M sodium acetate. The GEq/ml values of methylated cffDNA extracted using the THPG method were significantly higher than for the tested extraction methods ($p < 0.001$).

Mostafa Akbariqomi and Reza Heidari contributed equally to this work.

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